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REACTIONS OF CARBOHYDRATES WITH HYDROPEROXIDES

PART I. OXIDATION OF ALDOSES WITH SODIUM PEROXIDE*†

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ABSTRACT

It was found that aqueous alkaline hydrogen peroxide solutions at 0° degrade aldohexoses almost quantitatively to 6 moles of formic acid, and aldopentoses to 5 moles. A mechanism is proposed for the stepwise degradation of aldoses to formic acid, consisting of the addition of a hydroperoxide anion to the aldehydo modification of the sugar, followed by decomposition of the adduct to formic acid and the next lower aldose. The authors found that iron salts accelerate the reaction and suggested that decomposition of the peroxide adduct may take place by a free-radical process, as well as by an ionic mechanism.

Reactions of the following hexoses were measured: D-glucose, D-mannose, D-galactose, D-allose, D-altrose, and D-talose, and the following pentoses: D-xylose, D-arabinose, D-lyxose, and D-ribose. The reaction rates for the various sugars increased in the order just cited.

INTRODUCTION

Although the biological and technological importance of the reactions of carbohydrates with hydrogen peroxide and alkaline peroxides has been generally recognized, the reactions which take place are not clearly understood, and the results reported in the literature are highly confusing. The first systematic investigation of the oxidation of sugars under alkaline conditions was conducted by Nef[1–3], who found that D-glucose, D-mannose, and D-fructose, by reaction with oxygen or hydrogen peroxide, yielded formic and D-arabinonic acids, with small amounts of carbon dioxide, and D-erythronic, D,L-glyceric, glycolic, and oxalic acids. Nef considered the action of oxygen and peroxide to be essentially the same. His reactions were conducted at room temperature, and all of the reaction mixtures contained oxygen, introduced directly, or derived by thermodecomposition of hydrogen peroxide. In a concurrent investigation under Nef's guidance, Spoehr[4] studied the oxidation of D-glucose, D-fructose, and D-galactose with a higher concentration of alkaline per-

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*Dedicated to Professor V. Deulofeu, in honor of his 70th birthday.

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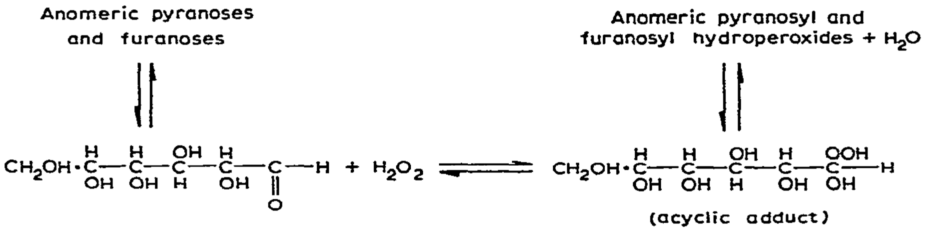
oxide. From D-glucose and D-fructose, under such conditions, he isolated principally formic acid, with smaller quantities of carbon dioxide, glycolic acid, and an acid subsequently identified by Nef[3] as D-arabinonic acid. Later, Bamford and Collins[5] found that D-glucose and D-fructose, in alkaline solutions, by treatment with oxygen under pressure, yield each approximately one mole of formic and D-arabinonic acids. The complex character of the reactions of carbohydrates with peroxides is pointed out by Moody[6] in a comprehensive review of the subject.

DISCUSSION AND RESULTS

A clear understanding of the separate reactions of the sugars with oxygen and with alkaline hydrogen peroxide is necessary to account for differences in the reaction products reported in the literature. To study the reactions of aldoses with the hydroperoxide anion, we selected conditions favoring this anion and suppressing the presence of oxygen, namely, low temperatures and high concentrations of peroxide. We found that under these conditions aldoses are oxidized almost quantitatively to formic acid. Thus, aldohexoses yield 6 moles of formic acid, and aldopentoses 5 moles (Table I). Aldopentoses react more rapidly than aldohexoses, and there is a considerable difference in the rates of reaction of the separate hexoses and pentoses. In the hexose series, D-glucose is oxidized most slowly, and D-talose most rapidly; in the pentose series, D-xylose is oxidized most slowly, and D-ribose most rapidly.

Proposed mechanism for oxidation of reducing sugars by alkaline hydrogen peroxide. — Nef[3] postulated that the products formed in the oxidation of hexoses arose by oxidative cleavage of the 1,2-, 2,3-, and 3,4-enediols of the sugar. Later workers[7] have followed this concept, with modification of Nef's mechanism for the oxidative cleavage of the double bonds. Our preliminary studies indicated that the rates of reaction of sugars with either oxygen or hydrogen peroxide under alkaline conditions are greater than the rates of enolization. Hence, enolization does not appear to be a prerequisite for the oxidation of the sugars with peroxide, and another oxidation mechanism is indicated.

Reducing sugars in alkaline solutions exist in several modifications capable of reacting with oxidizing agents. The species of the sugar that enters into any particular reaction depends on the character of the oxidant and the experimental conditions. Thus, bromine in slightly acid solutions attacks the pyranose form of an aldose having



Scheme I. Addition of hydrogen peroxide to D-glucose.

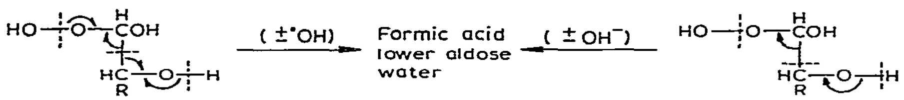
TABLE I

OXIDATION OF ALDOHEXOSES AND ALDOPENTOSES BY SODIUM PEROXIDE[a]

Reaction time (h)	*Hexoses*						*Pentoses*			
	D-*Allose*	D-*Altrose*	D-*Galactose*	D-*Glucose*	D-*Mannose*	D-*Talose*	D-*Arabinose*	D-*Lyxose*	D-*Ribose*	D-*Xylose*
20	3.05	4.33	2.42	1.00	1.81	5.01	3.28	4.14	4.84	2.91
46			4.75			5.86		4.91		
70	5.08	5.76			4.16					
93				2.74				5.04	4.97	4.74
120			5.63			6.04[b]	5.04[b]	4.94[b]	5.01[b]	4.95[b]
						6.06[c]	4.97[c]	5.02[c]	4.65[c]	4.89[c]
143	5.68	6.05			5.20					
284	5.89[b]	5.98[b]								
	5.61[c]	5.67[c]								
310	5.72	5.98			5.50					
360				4.86						
408			5.55[b]							
			5.55[c]							
480				5.30						
740				5.39[b]	5.95[b]					
				5.28[c]	5.84[c]					

[a] Moles of acid formed per mole of sugar; determined by titration of residual alkali, except for [b] and [c]. Reaction mixture: 75 mg of aldose in 1 ml of water plus 5 ml of M sodium peroxide containing 10 mg of EDTA, kept at 0° for times indicated. [b] Ether-soluble acid determined by titration. [c] Formic acid determined by reduction of mercuric chloride.

an equatorial anomeric hydroxyl group[8], whereas chlorous acid attacks the aldehydo form[9]. Because of the strong nucleophilic properties of the hydroperoxide anion[10], it seems probable that, in the peroxide oxidation of a reducing sugar under alkaline conditions, the reaction begins with addition of this anion to the aldehydo form of the sugar as depicted in Scheme I. Addition of the hydroperoxide anion yields an aldehyde peroxide analogous to an aldehyde sulfite or aldehyde hydrate. The proposed acyclic peroxide adduct may decompose by a free-radical mechanism such as that of Scheme IIa or by an ionic mechanism such as that of Scheme IIb. By either mechanism the products are formic acid and the next lower aldose. The lower aldose may then repeat the process with the result that the aldose is entirely degraded, stepwise, to formic acid.



IIa. Free-radical mechanism IIb. Ionic mechanism

Scheme II. Oxidative degradation of the acyclic adduct of an aldose.

The free-radical decomposition of the peroxide adduct shown in Scheme IIa begins with abstraction of the hydrogen of the hydroxyl group of C-2 by reaction with a hydroxyl radical. This is followed by rupture of the C-1–C-2 bond, with one electron going to form the carbonyl group of the next lower aldehyde, and the other electron to form the carbonyl group of formic acid, with regeneration of an hydroxyl radical. In the ionic mechanism shown in Scheme IIb, ionization of the hydrogen of the C-2 hydroxyl group provides a source of electrons for forming the carbonyl group of the lower aldehyde, with rupture of the C-1–C-2 bond. The electrons from this bond go to form formic acid by rupture of the oxygen–oxygen bond of the peroxide adduct, with release of a hydroxyl anion. The reactions may take place either by *trans* elimination as shown in the reaction scheme or by cyclic transition states.

In Scheme II, oxidative degradation of acyclic adducts is depicted. Pyranose and furanose hydroperoxides (presumably in equilibrium with the acyclic adducts) may give rise to similar reactions, with formation of formyl esters. Thus, as shown in Scheme III, D-glucopyranosylhydroperoxide would yield 4-*O*-formyl-D-arabinose, whereas D-glucofuranosylhydroperoxide would yield 3-*O*-formyl-D-arabinose. However, under alkaline conditions, these esters, if formed, would be hydrolyzed to the same products as those produced by the acyclic adducts.

Effects of the ferrous ion on the oxidation. — The oxidations reported in Table I were conducted under conditions selected to minimize the free-radical reaction. Thus, the solutions were maintained at 0° in the dark, and a small amount of ethylenediaminetetraacetic acid (EDTA) was added to inhibit the action of heavy metal catalysts. The experiments reported in Table II were conducted to show the effect of ferrous sulfate, a free-radical source, on the reaction rates.

TABLE II

EFFECT OF THE FERROUS ION ON THE OXIDATION OF D-GLUCOSE AND D-XYLOSE BY SODIUM PEROXIDE

Composition of reaction mixture[a]				*Acid formed*[b]											
Aldose (*g*)	Na_2O_2 (*g*)	*EDTA* (*mg*)	$FeSO_4$ (*mg*)	*20 h*	*28 h*		*78 h*		*168 h*		*336 h*		*700 h*		*Theory* (%)
				Total acid[c]	*Total acid*[c]	*Formic acid*[d]	*Total acid*[c]	*Formic acid*[d]	*Total acid*[c]	*Formic acid*[d]	*Total acid*[c]	*Formic acid*[d]	*Total acid*[c]	*Formic acid*[d]	
D-Glucose															
0.938	4.9				2.59	2.54			4.40	3.86			5.23	5.06	84.3[e]
0.938	4.9	125			2.47	2.28					5.20	5.03	5.46	5.15	85.8[e]
0.938	4.9		1.25		3.77	3.74			4.82	4.76	5.10	4.85	5.46	5.33	88.8[e]
D-Xylose															
0.938	4.9			3.31			4.91	4.72							94.4[f]
0.938	4.9	125		3.32			4.93	4.76							95.2[f]
0.938	4.9		1.25	4.86			4.96	4.73							94.6[f]

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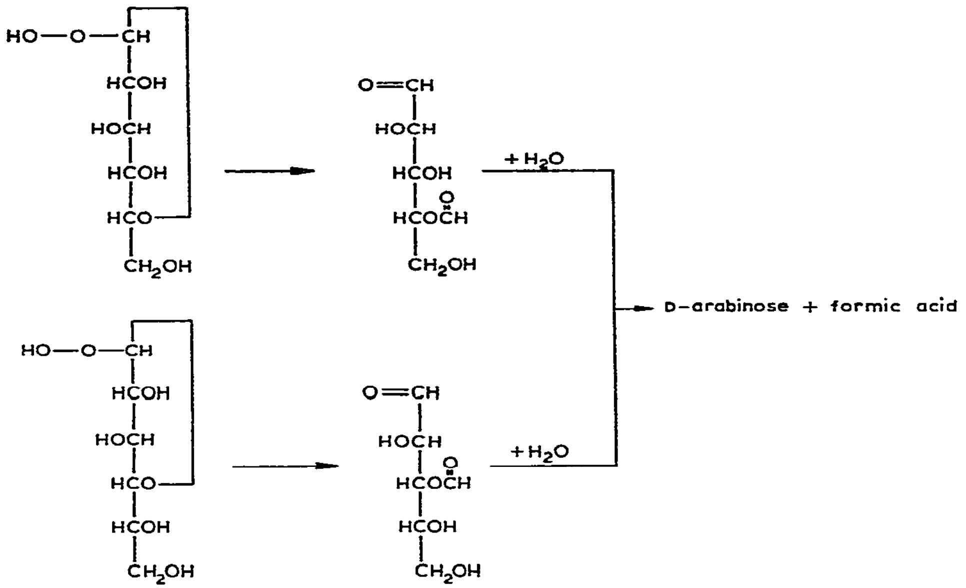
[a] Contents per 100 ml of reaction mixture. [b] Moles per mole of aldose. [c] Determined by titration. [d] Determined by reduction of mercuric chloride. [e] Based on formic acid found at 700 h, and a theoretical yield of 6 moles per mole of D-glucose. [f] Based on formic acid found at 78 h, and a theoretical yield of 5 moles per mole of D-xylose.



Scheme III. Oxidative degradation of pyranosyl and furanosyl hydroperoxides of D-glucose.

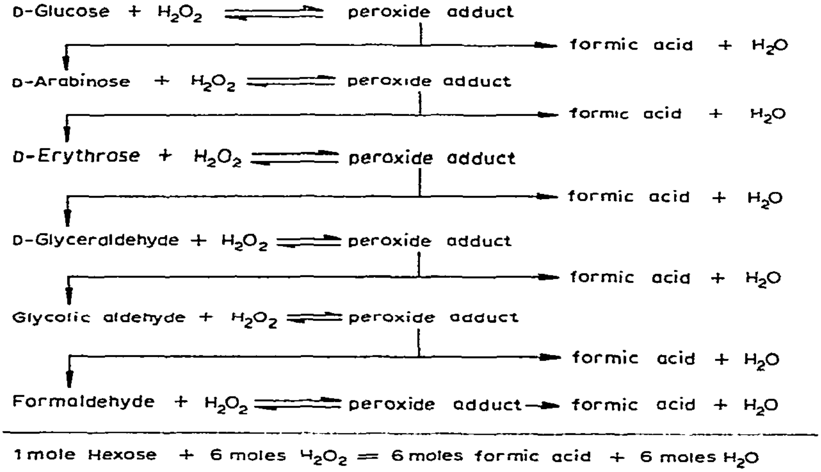
Hydrogen peroxide and ferrous salts in solution give the following reactions[11]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {\cdot}OH$$
$${\cdot}OH + H_2O_2 \rightarrow H_2O + {\cdot}OOH$$
$${\cdot}OOH + H_2O_2 \rightarrow O_2 + H_2O + {\cdot}OH$$
$${\cdot}OH + Fe^{2+} \rightarrow OH^- + Fe^{3+}$$

Solutions containing hydrogen peroxide and ferrous sulfate decompose spontaneously with evolution of oxygen gas. However, we found that with excess alkaline peroxide at low temperatures, the oxidation of aldoses proceeds smoothly to formic acid, even in the presence of ferrous salts. As shown by the results in Table II, the principal product of the reaction is formic acid, both in the absence of the ferrous ion and in its presence. The reaction is more rapid in the presence of ferrous sulfate, in agreement with the existence of a free-radical reaction such as that described in Scheme IIa. In complete absence of free radicals, the oxidation would be restricted to the ionic course of Scheme IIb. However, complete elimination of free-radicals in systems containing peroxides is not ordinarily realized. Presumably, the presence of the ferrous ion enhances the free-radical reaction, but at present we cannot distinguish quantitatively between the ionic and the free-radical course.

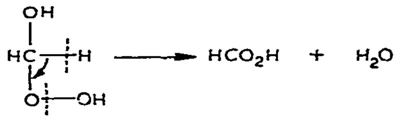
As seen in Table I, D-glucose is the least reactive of the aldoses studied. Even after more than 4 weeks the yield of formic acid, shown in Table II, had not reached 90% of the theoretical value. The ferrous ion, although expediting the reaction initially, did not appreciably alter the total oxidation. The sluggishness of the D-glucose reaction may permit unknown side reactions, with consequent decrease in formic acid.

The overall oxidation process. — The overall oxidation of D-glucose is outlined in Scheme IV. The system is complex, but provides an intriguing kinetic problem that deserves further investigation. The formation of the peroxide adduct from the aldehydo form of the sugar can be assumed to be rapid and reversible in alkaline solution, because of the similarity of this reaction to that of the formation of an aldehyde hydrate. The equilibrium proportion of the peroxide adduct will then depend on the characteristics of the sugar, especially its tendency to exist in the aldehydo form. Thus, glucose, which has the lowest proportion of the aldehydo form, has the lowest reaction rate of the six aldohexoses studied. In the pentose series, ribose, having the highest proportion of the aldehydo form, has the highest reaction rate. The peroxide adducts formed at each step of the overall reaction (except the last) are much alike, and presumably decompose by similar mechanisms at much the same rate. Epimeric hexoses, after the first degradation, give the same pentose; hence, the differences in the rates of reaction of epimers can be ascribed to differences in the rates of formation of aldehydo intermediates from the epimers, and conversion of these into the peroxide adducts.



Scheme IV. Proposed scheme for oxidation of D-glucose with alkaline hydrogen peroxide.

The oxidation of formaldehyde in the final step of the reaction may take place by either a free-radical or an ionic mechanism in the following manner:



EXPERIMENTAL

Oxidation of aldoses and determination of total acids formed. — Solutions used: (A) M Sodium peroxide containing 0.2% of EDTA; (B) aldoses, 7.5 g in 10 ml. Aliquots (1 ml) of solution B were placed in small tubes and treated at 0° with 5 ml of solution A. The tubes were stoppered and stored in an ice chest. At suitable intervals, the reaction mixtures were titrated with 0.1M hydrochloric acid (with Phenol Red indicator). Aliquots (5 ml) of solution A were similarly titrated. The net titration represented the total acid formed in the reaction.

Determination of ether-soluble acids. — Selected reaction mixtures were transferred to an ether extraction apparatus containing 15 ml of a cation-exchange resin (Amberlite IR-120-H). The acid in the ether extract was collected in 0.2M potassium hydroxide (15 ml). After an extraction period of 18 h, the alkali was back-titrated with 0.1M hydrochloric acid. The net titration represented ether-soluble acid, subsequently shown to consist almost entirely of formic acid.

Identification of formic acid by preparation of the p*-bromophenacyl ester.* — Selected samples of ether-soluble acids, after titration, were evaporated to dryness, and the *p*-bromophenacyl derivative was prepared by the method of Gabriel[12]. The crystalline ester was separated and recrystallized from 2-propanol. A similar preparation was made from pure sodium formate. The two products were shown to be identical by their i.r. spectra and R_F values on thin-layer chromatograms.

Determination of formic acid by the mercuric chloride method[13]. — The ether-soluble acid, after neutralization, was diluted to 100 ml. To an aliquot (30 ml) were added sodium acetate (5 g), acetic acid (5 ml), and mercuric chloride solution (40 ml of 5%). The mixture was heated on a steam bath for 2 h, and the precipitated mercurous chloride was collected on a weighed filter which was dried to constant weight at 100°. A small correction was made for a blank determination. Formic acid = $Hg_2Cl_2 \times 0.0977$.

Determination of the effect of EDTA and ferrous ion. — Solutions: (A) Aldoses (D-glucose or D-xylose), 7.5 g in 100 ml; (B) EDTA, 0.5%; (C) ferrous sulfate, 5 mg in 100 ml; (D) M sodium peroxide.

Reaction mixtures were prepared from 10 ml of solution A, 20 ml of water or solution B or C, and 50 ml of solution D; reagents were added in the order cited. Mixtures were kept in the dark at 4°, and aliquots (10 ml) were analyzed at the times indicated in Table II. Total acid and formic acid were determined by the procedures previously described.

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